release of cartilage matrix degrading enzyme MMP-3 (stromelysin 1).

Conclusions: Our findings suggest a novel mechanism by which synovial cells induce degradation of cartilage matrix through SDF-1 signaling in rheumatoid arthritis and osteoarthritis.

PP29

IN VIVO INTERLEUKIN-1 CONVERTING ENZYME MODULATION BY NITRIC OXIDE IN THE DOG EXPERIMENTAL OSTEOARTHRITIS MODEL

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Aim: The aim of this study was to investigate the relationship between two key mediators implicated in cartilage osteoarthritis (OA): nitric oxide (NO) and interleukin (IL)-1 converting enzyme (ICE). IL- 18 was also studied and served as reference to the effect of ICE.

Method: For this study, an OA model was created by sectioning the anterior cruciate ligament (ACL) of the right stifle joint of dogs by a stab wound. Dogs were separated into experimental groups: I) unoperated dogs that received no treatment; II) operated dogs (OA) that received no treatment, and III) operated dogs (OA) that received oral N-iminoethyl-L-lysine (L-NIL, a specific iNOS inhibitor; 10 mg/kg/day) starting immediately after surgery. The OA dogs were killed 12 weeks after surgery. In a second set of experiments, ACL was induced in dogs for 12 weeks, and femoral condyles dissected and incubated with specific inhibitors of important signaling pathways involved in the OA process, namely SB 202190 (10 mM, p38 MAP kinase inhibitor), PD 98059 (100 mM, MEK 1/2 inhibitor), NS-398 (10 mM, specific cyclooxygenase-2 inhibitor), SN-50 (50 mM, NF-kB inhibitor) and L-NIL (50 mM). Specimens were processed for in situ hybridization or immunohistochemistry for ICE and IL-18, followed by a morphometric analysis.

Results: Data showed that ICE and IL-18 were both present in vivo in dog cartilage. Compared to normal, their levels were significantly increased in the OA dog cartilage. L-NIL treatment induced a decrease ($p<0.001$) of ICE and IL-18 levels throughout the OA tissue for both the femoral condyles and tibial plateaus; values were similar to normal dogs. This effect, however, appeared to occur post-transcriptionally as L-NIL did not affect ICE mRNA levels as demonstrated by in situ hybridation. Interestingly, in vitro experiments demonstrated a significant inhibition of the ICE levels by the above inhibitors except L-NIL, indicating that in vivo the NO effect is not direct but requires intermediaries.

Conclusion: In this study, we demonstrated the in vivo effectiveness of a specific NO inhibitor in completely reversing the OA enhanced ICE and IL-18 synthesis levels. Data further suggests that the in vivo NO inhibition of ICE levels requires intermediary factors.

PP30

GENE TRANSFER INTO THE PERIOSTEAL GRAFTS FOR ARTICULAR CARTILAGE DEFECTS USING GENE GUN SYSTEM

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Aim: The aim of this study was to examine expression rate and duration of LacZ marker gene transfected by gene gun and to analyze tissue damage of the periosteal grafts.

Methods: Twelve adult male New Zealand White rabbits weighing average 3.20kg were used. Periosteal grafts were harvested from the medial proximal tibia and LacZ marker gene (pCMV b) was transferred into cambium layer of the grafts by gene gun. The grafts was placed on the articular cartilage defects on the femoral component of patellofemoral joint with the cambium layer facing down on the articular surface. LacZ gene transfer was not performed in the control grafts. The animals were sacrificed at 2,4 and l2 weeks after operation and the knee joints were harvested for gross macroscopical observation, followed by histopathological examination. The newly formed tissue was assessed by the semiquantitative histological grading system (0~l4 points), and the efficiency of gene transfer was calculated by counting the number of total cells and LacZ positive cells in the central area.

Results: The total histological score of repair tissue at 2 weeks was 11.3 ±l.3 (mean±SD) points in the LacZ group and 11.5 points in the control group. At 4 weeks, it was 5.6±1.1 in the LacZ group and 5.3± l.2 in the control (low score indicating better cartilage repair). These results indicates that the gene gun does not affect the tissue repair process of periosteal grafting.

The average LacZ positive rate was 13.0% at 2 weeks and 8.9% at 4 weeks.

Conclusions: The expression of LacZ was observed at 4 weeks after grafting with neither inflammation nor tissue damage. The in situ gene delivery system using gene gun seems to be suitable for gene therapy in the Orthopaedic field since it has less risk of systematic effects than in vivo methods with viral vectors, it is easier to perform than ex vivo methods, and it can provide local transfection during surgery

PP31

INTEGRIN-MEDIATED MAP KINASE SIGNALING INCREASES COLLAGENASE-3 (MMP-13) PRODUCTION BY HUMAN ARTICULAR CHONDROCYTES

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Aim: The aim of this study was to determine if integrin-mediated signaling results in activation of chondrocyte mitogen-activated protein (MAP) kinases which lead to increased expression of MMP-13 (collagenase 3), a potent mediator of cartilage matrix degradation.

Methods: Human articular chondrocytes in primary confluent monolayer cultures were treated with integrin blocking antibodies and fibronectin fragments. MAP kinases were analyzed by immunoblotting with phospho-specific antibodies and MMP-13 production was measured in conditioned media by immunoblotting and with a peptide substrate based assay.

Results: Chondrocytes were found to respond to the 120 kDa fibronectin fragment and to blocking antibodies to the $\alpha2\beta1$ and $\alpha5\beta1$ integrins with increased phosphorylation of the ERK1, ERK2, JNK, and p38 MAP kinases. Intact fibronectin and block-

ing antibodies to α1, α3, and αVβ3 integrins as well as a non-blocking α5β1 antibody had no affect. MAP kinase activation was associated with increased phosphorylation of pyk2 but not focal adhesion kinase. Subsequent to MAP kinase activation increased phosphorylation of c-Jun and the NFkB inhibitor 1kB was noted, followed by increased pro- and activated MMP-13 in the conditioned media. Inhibitors of MEK and p38 were both able to inhibit the increased MMP-13 production while the interleukin 1 receptor antagonist protein did not. However, the IL-1 receptor antagonist partially inhibited fibronectin fragment induced activation of MMP-13.

Conclusion: These results suggest that disruption of chondrocyte binding to a native matrix by a5b1 blocking antibodies or fibronectin fragments stimulates integrin-mediated MAP kinase activation resulting in increased production and release of pro- and active MMP-13. Autocrine production of IL-1 results in additional MMP-13 activation.

PP32

ONCOSTATIN M INDUCES COLLAGEN DEGRADATION IN HUMAN ARTICULAR CARTILAGE

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Aim: This study investigated the effect of oncostatin-M (OSM) on collagen degradation in explants of human articular cartilage.

Methods: Monoclonal antibody (mAb) 2B4 was raised against a sequence from the C-telopeptide cross-linking domain of type II collagen and recognizes a neoepitope of matrix metalloproteinase cleavage. Full thickness plugs of human articular cartilage from the tibial plateau (banked tissue) or femoral condyle (surgical amputation) were cultured in DMEM at 37°C in the presence or absence of recombinant human OSM (50 ng/ml) for up to 4 weeks. Conditioned media were collected every 7 days and assayed for collagen fragments by competition ELISA based on mAb 2B4. Cartilage plugs were recovered after 1, 3 or 7 days of culture for immunohistochemical analysis using mAb 2B4.

Results: Assay of the culture media showed elevated levels of 2B4 epitope in OSM-treated cartilage plugs after 14 days of culture (OSM-treated 29.8 ± 5.3 ng/ml vs control 15.5 ± 3.4 ng/ml, P = 0.006). By day 28, medium 2B4 epitope levels reached 208.0 ± 102.0 ng/ml for OSM-treated plugs compared with 39.0 ± 7.0 ng/ml for control plugs (P = 0.045). Cartilage plugs analyzed for 2B4 epitope by immunohistochemistry showed strong pericellular staining in plugs treated with OSM for 3 to 7 days. The 2B4 signal was virtually absent from control plugs cultured without OSM, and from plugs cultured with OSM for less than 3 days.

Conclusion: OSM, a proinflammatory cytokine found in high levels in human rheumatoid synovial fluids, induces collagen degradation in human articular cartilage *in vitro*. Degraded collagen fragments are seen in the tissue after 3 to 7 days of OSM-treatment, sooner than reports of collagenase upregulation following cytokine stimulation. The findings support a concept that telopeptidase activity initiates collagen fibril degradation in this system.

PP33

ATP INDUCED-PROSTAGLANDIN E2 SYNTHESIS IS MEDIATED BY ERKI/2 AND P38 MAP KINASES VIA P2Y RECEPTORS IN ARTICULAR CHONDROCYTES

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Aim: The aim of this study was to assess the effects of ATP on the secretory profiles of prostanoids and the coupled signaling and enzymatic events in primary cultured monolayers of rabbit articular chondrocytes. ATP is an inflammatory mediator that is released from immune cell activation and damaged tissue at sites of inflammation.

Results: ATP increased PGE2 release in a dose dependent fashion, with an activation threshold at 10 mM ATP and a 4.7-fold increase in PGE2 output at 100 mM ATP. ATP-induced PGE2 was detectable as soon as 2 min, and reached maximum level at 1 hour. Purinoceptor agonists stimulated PGE2 release by the chondrocytes with the following rank order of potency ATP = UTP ATPgS > ADP. Adenosine was ineffective. The P2 receptor selective antagonists suramin and reactive blue-2 inhibited ATP-induced PGE2 release. These results strongly suggest that ATP causes the release of PGE2 via the stimulation of P2Y receptors. Furthermore, extracellular nucleotides stimulated a rapid, transient and concentration-dependent activation of stress- and mitogen activated protein kinases (SAPK and MAPK), respectively p38 and ERKI/2, as measured by phosphorylation of the both kinases. The activation of p38 kinase and ERKI/2 by ATP and UTP was dose dependently attenuated by suramin.

To understand the enzymatic cascade implicated in the ATP dependent PGE2 release, we first tested AACOCF3, an inhibitor of cPLA2 and BEL, an inhibitor of the iPLA2. The response induced by ATP was not affected by BEL and completely blocked by AACOCF3, suggesting that cPLA2 mediated the release of PGE2 in response to purinergic agonists. A complete blockade of the ATP-induced PGE2 release was observed with each inhibitor of the MAPKinases PD *98059* and 5B203580. In contrast, the release of arachidonic acid in response to ATP was only affected by PD 98059, suggesting that cPLA2 activation was mediated through activation of the ERKI/2 MAPKinase. The following enzymatic events were investigated with the use of NS-398, a selective COX-2 and PGE-synthase inhibitor and SC-560, a selective COX-1 inhibitor. Both of them prevented ATP-induced PGE2 release.

Conclusion: We conclude that extracellular ATP, acting via P2Y-purinoceptors, stimulates PGE2 release from articular chondrocytes and thus may have physiological and pathological effects in articular cartilage.

PP34

SEQUENTIAL CLEAVAGE OF AGGRECAN IN BOVINE ARTICULAR CARTILAGE BY HUMAN RECOMBINANT AGGRECANASE MIMICS INTERLEUKIN-1 INDUCED CARTILAGE TURNOVER

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Aim: The aim of this study was to track the process of aggrecanolysis in intact bovine articular cartilage (BAC) induced by recombinant human interleukin-1b (IL-1) or by the action of recombinant human aggrecanase-1 (rhAgg-1) *in vitro* using immunocytocheimcal methods.